COMMONWEALTH OF PENNSYLVANIA
PENNSYLVANIA FISH & BOAT COMMISSION

ADMINISTRATIVE POLICY

SUBJECT: Biosecurity Measures for Commission Operations, Facilities, and Equipment

NUMBER: 2009-001

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In recent years, introduction of various aquatic invasive species (AIS) into the waters of the Commonwealth, and areas hydrologically connected to Pennsylvania, have been well-documented. AIS include both microscopic and macroscopic organisms, with highly varied distributions. Some macroscopic AIS, such as zebra mussels (*Dreissena polymorpha),* Didymo (*Didymosphenia geminata),* and northern snakehead (*Channa argus),* are already found in Pennsylvania, whereas other species (e.g., bighead carp, *Hypophthalmichthys nobilis,* and silver carp, *H. molitrix*) are in the Ohio River and are expected to eventually reach waters of the Commonwealth. The microscopic AIS, Viral Hemorrhagic Septicemia IVb (VHS) virus, has been identified in the Great Lakes Basin and its occurrence may have widespread implications for wild and hatchery fishes and the aquaculture industry. These organisms pose potentially significant ecological and economic threats to Pennsylvania. For fish production, AIS can pose a serious health issue for reared fish as well as having substantial economic implications for the Commission. In fragile ecosystems, AIS may compete with, or prey upon, native flora and fauna.

To reduce the threat presented by AIS, the Commission has developed the attached protocols for its field operations, fish production, and disease monitoring procedures. These procedures will be implemented to reduce the inadvertent transmission of AIS, especially as a result of activities that require staff to regularly enter or move equipment and materials between water bodies. Commission staff will, to the extent practical, follow the most current protocols for disinfecting equipment and other items moved between waters of the Commonwealth.

This policy remains in effect until revised or rescinded by the Executive Director.

Pennsylvania Fish and Boat Commission Biosecurity Protocols:

Procedures to minimize the transfer of aquatic invasive species into or between waters of the Commonwealth

Table of Contents

1. Introduction

In recent years, potential environmental problems associated with the introduction of various aquatic invasive species (AIS) have become well-known. The effects of some of these organisms are well-documented. Zebra mussels (*Dreissena polymorpha)* are already present in waters where Pennsylvania Fish and Boat Commission (PFBC) staff operate boats. Didymo (*Didymosphenia geminata)* was recently discovered in the upper portion of the Delaware River and in the Gunpowder River in northern Maryland. Additionally, Viral Hemorrhagic Septicemia IVb (VHS) virus has been identified in the Great Lakes Basin and the efforts to control its spread have had widespread implications for the aquaculture industry. The effects of other, lesser-known AIS are only beginning to be understood. As an example, chytrid fungus (*Batrachochytrium dendrobatidis*), of African origin, is a globally decimating amphibian species and would be a serious threat to Pennsylvania species if it becomes established. Overall, AIS pose threats to the ecological health and the economic benefits of the waters of the Commonwealth, to the state's natural biodiversity, to the operation of PFBC facilities, and to the agency's ability to fulfill its mission. The PFBC has enhanced its fish production and disease monitoring procedures to address this problem. However, additional procedures are needed to further minimize the possible inadvertent spread of AIS through routine PFBC activities which require staff to regularly move boats, sampling equipment, and other items between water bodies. This document establishes procedures to be implemented by PFBC field staff to help prevent the spread of aquatic invasive species and/or other potentially harmful aquatic organisms. The following procedures must be followed when fieldwork necessitates the movement of boats and equipment between waterways or across watershed basins. To the extent practical, all susceptible equipment moved between watersheds must be properly cleaned and disinfected. Particular attention must be given to situations where AIS are known or suspected to occur. These guidelines were developed, in part, from biosecurity protocols currently being used in Wisconsin and New York. Additional information used in the development of this document was obtained from http://www.biosecurity.govt.nz/ and http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/specimen_collection.jsp.

2. Surveys and Sampling Guidance

A. It is assumed that all waterways and all locations within a given watershed are vulnerable to AIS infestation. Therefore, to minimize and avoid transport of AIS as a result of Commission activities, only properly treated equipment shall be used during activities conducted in waters of the Commonwealth. It will be the responsibility of all PFBC field staff to stay current with any announced changes to this Biosecurity Protocol.

B. For the purposes of this document, hatchery waters are considered waters of the Commonwealth. Hatchery protocols are discussed later in this document. All vehicles and boats entering the hatchery areas must be thoroughly disinfected following the appropriately prescribed protocols described throughout this document. Protocols for each hatchery may need to be developed to address circumstances unique to each facility. Personnel must follow hatchery-specific biosecurity procedures when conducting sampling or marking hatchery fish.

C. For the purposes of this document, wetlands, vernal pools, and similar amphibian and/or reptile habitats are considered waters of the Commonwealth. It is critical that biosecurity protocols, particularly those pertaining to the spread of disease pathogens, be followed when equipment is exposed to or transported between these waters. The below disinfection procedures, particularly those involving the use of chlorine bleach, are effective against the pathogens of concern.

D. As part of the routine scheduling of any PFBC activity that will occur on waters of the Commonwealth or waters located in neighboring states or countries, every reasonable effort will be made to determine if AIS occur in those waters. This will allow precautionary measures to be taken to prevent translocation of AIS into non-infected waters or transmission from infected waters. Depending on the type of work being done, it may be possible and desirable to work with other agencies or partners to use equipment located on-site to collect samples. This would potentially limit the amount of equipment required for disinfection.

E. The Commission shall provide extra equipment to ensure that disinfected or dry equipment is available. If having duplicate gear items is not practical, then all susceptible equipment must be properly treated prior to use. In situations when activities are scheduled to occur in succession on both infected and non-infected waters, then non-infected waters must be worked prior to working infected waters. **Do not work infected waters first!**

F. If a high percentage of work activities are done in waters with AIS, staff shall dedicate certain equipment for use only in those waters.

G. For activities conducted in waters of the Commonwealth where the status of AIS is unknown, work shall start at the upper-most reach and then proceed in a downstream or down lake direction, if feasible. This will ensure that non-motile organisms are not transported on boots or other equipment to uninfected up-stream or up-lake locations.

H. If a water of the Commonwealth is known to contain AIS, but the extent of infestation is not clear, then efforts shall be made to replace or disinfect equipment before beginning subsequent activities.

I. In waters of the Commonwealth where occurrences of AIS are known to be system-wide, work order and preventative measures are less important. It must not be assumed, however, that all waterways within a watershed are infected. When in doubt, disinfection procedures shall be followed when moving between waterways.

J. If a new occurrence of an AIS is suspected, the following steps shall be taken:
   1. Document the location of the suspected AIS. (Collect GPS coordinates if possible.)
   2. If possible, secure a specimen for positive identification by qualified personnel. (Fisheries Management staff shall have specimen collection kits available on all surveys.)

3. If specimen collection is not possible, secure a high-quality digital image or color photograph.
4. Notify appropriate PFBC staff: Communications Chief, Bureau Director-Fisheries, Chief of Fish Production, Chief-Fisheries Management, Bureau Director-Boating and Access. No information should be released to the public until a positive identification of specimens is verified.

3. Exposure and Handling of Diseased Specimens

A. Causal assessment of external abnormalities or death in amphibians, reptiles, or fish

First, note whether there are sick, deformed, or dead animals of more than one vertebrate class and phyla (e.g., dead birds, frogs, fish, snails, insects) present in the immediate area; if so, there is a much greater chance the problem was caused by a toxicant (poison). In this case, field personnel should exercise caution to prevent self-contamination. If, however, only one taxon (type of animal) has been affected, it is more likely that the illness, deformities, or deaths are due to an infectious disease.

B. Disease precautions and procedures

Any amphibians, reptiles, or fish (dead or alive) that appear to be "sick" or deformed should be considered contagious specimens. Only handle suspected animals while wearing "rubber" gloves. Dispose of the gloves after handling the animal and do not use them to handle other reptiles, amphibians, or fish at the site. Retained specimens are to be secured in appropriate containers such as tightly capped bottles or doubled zip bags, immediately labeled (date, place, etc.), and the exterior of the container is to be disinfected. Specimen kits appropriate for collecting potentially contagious specimens will be made available to all field units. Affected living animals and any carcasses should never be released or discarded at other sites and should not be taken into laboratory settings with other amphibians, fish, or reptiles. Follow the disinfection guidelines below for any exposed equipment. Contact the Natural Diversity Section (814-359-5237) for further instructions for disposal or transport (for testing, identification, etc.) of the diseased specimen(s).

4. Equipment Disinfection Protocols

These protocols are to be used to reduce the risk of spreading AIS during all Pennsylvania Fish and Boat Commission activities.

A. Boat and Trailer

1. Upon arrival and prior to launching, and upon removal from the water and prior to departure from a boat launch site, the following procedures will be conducted:

a) Inspect and remove all visible aquatic plants, animals, mud, and other organic material from the boat, trailer, and equipment at the work location. Aquatic plants, animals, mud, and other organic material found on equipment prior to launching that remained from a previous

location shall be collected and placed into an approved container for transport back to regional offices for proper disposal.

b) Drain the bilges of the boat by removing the drain plug. Bilge pumps are not capable of removing all water from those areas. Wet wells, live wells, and any other compartments that could hold water must be drained of water at the field site, when appropriate. If the boat and trailer will not be in contact with other waters of the Commonwealth, the bilge area may be drained upon return to the boat storage facility provided that facility is sufficiently isolated from local waters and hatchery operations as to prevent their contamination.

c) Disinfect trailers equipped with carpeted bunks after the boat is launched, when the boat is not being returned to the trailer, *and* the trailer is being removed from the launch site. The trailer may be decontaminated at the storage facility if there is no potential for contaminating other waters. Disinfect the trailer according to one of the approved methods described in Appendix 1.

2. Upon return to the storage facility and prior to launching into another water of the Commonwealth:

a) Inspect and remove any remaining aquatic plants, animals, mud, and other organic material from the boat, trailer, and equipment at the work location and dispose of properly.

b) Recheck the bilges, wet wells, live wells, and any other compartments for any remaining water. Spray these areas.
   i. If bilge water is drained at the storage facility, the water shall be collected, disinfected, and disposed of properly to avoid causing environmental damage or contamination.
   ii. Pumps must be operated to take in the disinfectant and make sure that the solution comes in contact with all parts of the pump and hose.

c) After draining all water from boat compartments, all compartments that held water shall be washed with a high temperature (200^{o}F) pressure washer or with an approved disinfectant and allowed to remain wet for the appropriate contact time, as described in Appendix 1. Compartments shall be left open to completely dry prior to next use.

d) All boats and trailers used in field activities will be cleaned using a high temperature pressure washer or through application of disinfectant solution working from fore to aft and gunnels to keel in a thorough manner.
   i. Particular attention must be paid to the cooling water intakes on the lower unit of the motor.
   ii. Particular attention must be paid to the carpeted trailer bunks since they can hold water for extended periods of time.

iii. Lower the motor to drain all water from the lower unit and disinfect motor according to the procedures described below.

e) After application of disinfectant solution, the boat, trailer, bilges, live well, and pumps must be rinsed with clean water after the appropriate contact time. ***Every effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solutions.***

B. Boat Motors

1. Upon return to storage facility, and prior to launching into another water of the Commonwealth, boat motors shall be treated in the following manner:

a) Outboards

i. Clean all exterior parts of the motor with one of the approved methods described in Appendix 1.
ii. Immerse the lower unit in a bucket of disinfectant and run the motor to ensure contact with all internal parts allowing for appropriate contact time as described in Appendix 1.
iii. Attach a short (6-foot) piece of garden hose to lower unit muffs. A pail of the disinfectant can be set in the back of the boat and gravity fed to the lower unit to run the disinfectant through the motor. The hose will need to be primed to start the gravity flow because the lower unit does not create enough suction to prime the hose.
iv. Allow the disinfectant to remain in the motor for the appropriate contact time.
v. A non-corrosive disinfectant such as *Virkon Aquatic* is recommended for use to protect the impeller. PLEASE NOTE: Virkon Aquatic® is labeled for use only as a bactericide and viricide! Do not depend on its use against other AIS such as invertebrates (e.g., zebra mussel), plants, vertebrate species, etc. See Appendix 1 for other disinfection methods!

b) Jet Drives

i. Clean all exterior parts of the motor with one of the approved methods described in Appendix 1.
ii. Spray any open and accessible portions of the water intake and nozzle portions of the motor. A non-corrosive disinfectant such as *Virkon Aquatic* is recommended. PLEASE NOTE: Virkon Aquatic® is labeled for use only as a bactericide and viricide! Do not depend on its use against other AIS such as invertebrates (e.g., zebra mussel), plants, vertebrate species, etc. See Appendix 1 for other disinfection methods!

2. After application of disinfectant solution, the motor must be rinsed with clean water after the appropriate contact time. ***Every effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solution.***

C. Commonly Used Equipment

1. After use, and prior to using equipment in another water of the Commonwealth, the equipment must be treated using the following procedures. Careful record keeping and equipment labeling will be necessary to ensure that equipment has been treated for sufficient time with the proper disinfection procedures and to ensure that dedicated equipment will only be used in its assigned waterways.

a) Large Equipment (e.g., stocking trucks, dredges) – Organic debris must be removed prior to disinfection. Power washing is not required, but large equipment could be sprayed with a garden hose to remove debris. Equipment may be steam cleaned, washed, and dried thoroughly for five days or treated with a disinfectant. When appropriate, immerse equipment in disinfectant for the required contact period as described in Appendix 1.

i. After application of disinfectant solution, the equipment must be rinsed with clean water after the appropriate contact time. ***Every effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solution.***

b) Small Equipment (e.g., buckets, water sampling equipment, electrofishing equipment) – Remove all organic material from gear and follow ***one*** of the options described below.

i. Spray with disinfectant and maintain a wet surface for the appropriate contact time described in Appendix 1.
ii. Fill a tub with disinfectant and place all equipment in the tub for the appropriate contact time as described in Appendix 1.
iii. Use a completely new set of equipment for each water body sampled throughout the work day or work week. Disinfect all equipment at the end of the activity using option one or two.
    - Dissolved oxygen probes and other sensitive electronic equipment can be damaged by disinfectants and must only be rinsed with clean water. Do not store dissolved oxygen probes or other water chemistry gear in water from the work site. Use distilled or tap water for probes and empty all lake containers and samplers used during chemical or vertical profile assessments at the survey location.

c) Personal Protective Equipment (e.g., rain gear, gloves, boots, waders, and PFDs) – Remove all organic material from gear and follow ***one*** of the options described below.

i. Scrub personal protective equipment with an approved disinfectant. After scrubbing, the equipment must be kept wet with the disinfectant for the appropriate contact time as described in Appendix 1.
ii. Personal equipment may be steam cleaned or dried thoroughly for five days after cleaning with soap and water.
iii. After application of disinfectant solution, the equipment must be rinsed with clean water after the appropriate contact time. ***Every effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solution.***

5. PFBC State Fish Hatchery (SFH) Protocols

Recent outbreaks of Viral Hemorrhagic Septicemia (VHS) in the Great Lakes drainage have drawn attention to the need for biosecurity improvements to prevent or minimize the possible introduction of VHS and other pathogens and aquatic invasive species into our fish production facilities. The needs and abilities of individual production facilities to adopt biosecurity measures will vary and a "one size fits all" approach is not practical, but both short- and long-term efforts must be directed at improvement. In general, each hatchery must be evaluated and, within each hatchery, zones of high and low disease risk must be identified. Each identified zone should have its own equipment and specific zone isolation and disinfection procedure. The following are areas for consideration when developing individual hatchery biosecurity plans.

A. Hatchery Water Sources

Optimally, water sources should be PFBC-owned, fenced, and free of fish. Water sources need to be as secure as possible within the parameters of each hatchery. Minimally, springs and wells must be fenced and secured where feasible.

1. Hatch Houses
   a) Hatch house influent water – Most facilities have equipment for UV treatment and pre-filtration of hatch house influent water. In some cases, it is limited to egg incubators only. As funding becomes available, these systems should be upgraded to include all hatch house production water. These systems must be properly maintained, including the cleaning of quartz sleeves and the replacement of UV bulbs at manufacturer recommended periods. Where feasible, upgrading to ozone treatment should be considered.

   b) Egg disinfection – It is important that all production trout eggs be properly disinfected. The modified California method has been tried at many of our trout stations without any significant additional egg mortality. This procedure triples the standard surface disinfection contact time and, at least theoretically, allows iodophor to enter the egg during the hardening process. This procedure must be made a Standard Operating Procedure (SOP) at all trout hatcheries. All eyed eggs should be processed through a mechanical picker to remove dead eggs and then surface disinfected with iodophor before further incubation. Eggs shipped to other SFHs must be disinfected by the receiving facility before being placed into incubation units. Warm/cool water eggs must also be disinfected per instructions from Production Managers based on the results of ongoing egg disinfection studies.

   c) Hatch house equipment – Hatch house equipment (nets, brushes, buckets, basins, etc.) must be dedicated for hatch house use only (color-coded) and this equipment must be stored away from the equipment used in outside rearing units. Equipment disinfection containers that are sufficient in depth to submerse nets, brushes, etc., must be present in all hatch houses. These containers must be properly maintained with disinfectant to ensure complete

disinfection. Nets, brushes, and other equipment must be allowed a sufficient contact time for complete disinfection. Rearing units should be surface disinfected between lots of fish. Suitable disinfectants may include *Virkon Aquatic* or *Iodophor* solutions, depending on use.

d) Spawning – During spawning activities, brood fish should not be brought into hatch house areas where eggs or juvenile fish are cultured. Disinfectant footbaths must be used when transporting eggs into hatch house/egg incubation areas. If fish must be spawned in proximity to hatch house eggs/fish due to hatchery limitations, a specific spawning area with proper disinfection and isolation must be set up to minimize contamination. Only disinfected materials (e.g., eggs, equipment) are allowed to leave this area and enter other areas of the hatch house. The spawning area must be thoroughly disinfected at the end of each day.

e) Cleaning activities – All rearing units should be cleaned daily. All nets, brushes, and other equipment, especially mortality collection nets, must be disinfected between each tank or rearing unit. As indicated above, sufficient contact time must be permitted for disinfection of the equipment. All mortalities should be removed from rearing units daily, and they must be disposed of properly. Mortalities must not be disposed of in tank drains or in open drain aqueducts.

f) Access to hatch house buildings – Access to hatch house buildings should be restricted to essential staff only. All equipment brought into hatch house buildings must be surface disinfected. All staff must use disinfectant footbaths and wash their hands with disinfectant soap before entering a hatch house building. Hatch houses with garage doors or multiple-use should have a barrier (e.g., simple hanging chain) around tanks to force foot traffic through foot bath areas. These areas may be off-limits to visitors and tour groups.

2. Outside Rearing Units

a) Influent water disinfection – Systems to disinfect influent water for outside rearing units are currently not available in PFBC hatcheries. As funding becomes available, case-by-case consideration should be given to installing such systems.

b) Cleaning activities – Solids collection sections of rearing units must be cleaned regularly according to best management practices. Mortalities should be removed daily from the entire rearing unit, not just at the effluent rack or screen. Unless untreated, recirculated water is used, rearing units should be cleaned and mortalities should be removed in a downstream progression by row, not across rows. Exceptions may be made for limited water flow conditions that would harm the fish by cleaning as stated above. Dedicated sets of nets, brushes, and other equipment must be provided for the raceway area.

c) Predator control – Where available, bird netting and other predator controls must be maintained and operated properly to prevent the entry of predators into rearing units.

d) Brood fish – Brood fish should be held in rearing units that are isolated from production fish. If this is not possible, brood fish should be held at the heads of raceway rows or in rows dedicated to brood fish only and physically separated from adjacent rearing unit rows. Brood fish should not be held in recirculated water.

e) Aqueducts and piping – At least annually, efforts must be made to eliminate escapee fish from pipes and aqueducts. These fish serve as reservoirs for fish pathogens. Escapee fish in downstream piping and polishing ponds should never be moved into upstream rearing units. Open aqueducts should be cleaned at least annually to remove aquatic vegetation and accumulated debris.

f) Cool/warm water areas – Dedicated, color-coded equipment (nets, brushes, etc.) must be maintained for use in outdoor cool/warm water rearing areas. Combination hatcheries (cold-ww/cw) must have separate equipment for both outdoor rearing areas. All potential means of cross contamination between cool/warm water culture areas and coldwater culture areas must be avoided.

g) Hand feeding – Employees engaged in hand feeding must ensure that scoops or other utensils are used to distribute the feed. In instances where utensils are not used, hands should be covered by gloves that are dedicated for use at a specific feeding site or rearing unit.

B. Stocking Procedures

Necessary precautions need to be taken to minimize contaminating stocking equipment (nets, buckets, hoses, etc.) during stocking operations. Waterways Conservation Officers shall inform volunteers to keep buckets out of the receiving waters and dump buckets of fish rather than dip them into waters. Stocking buckets shall be labeled "Dump It Don't Dip It" using commercially available stencils and permanent ink or paint. If volunteers contaminate a bucket, they should be given another bucket if available. Contaminated buckets and equipment must not come into contact with uncontaminated hatchery water within the transport tanks. Any contaminated equipment shall be disinfected by drivers before going to another water body for stocking. A spray bottle pre-mixed with disinfectant will be acceptable for most applications. Disinfected buckets shall be rinsed with transport tank water prior to adding more fish to the bucket. In situations where the transport tank water is tempered by stream or lake water to reduce fish stress and buffer temperature changes, bucket disinfection should be done and rinse water taken from tank compartments that were not tempered before going to another water body. When tempering is necessary, all tempered tank water will be discharged from the stocking truck before moving on to the next water body or returning to the hatchery. Additional disinfection measures should be taken when returning to the hatchery as outlined elsewhere in these protocols.

C. Trucks and Other Equipment

All vehicles and their equipment, including stocking trucks, boats, boat trailers, sludge trucks, construction and maintenance equipment, and other vehicles that contact water bodies outside

of a specific fish production site must be disinfected prior to entering the fish production portion of a hatchery. If stocking trucks and associated equipment (e.g., nets, buckets, hoses) come into contact with receiving waters, equipment must be disinfected before moving to the next water body for stocking purposes. All containers and other equipment used to transport fish, fish gametes, or fertilized eggs to or from other facilities, or used by other facilities must be disinfected and all associated transport water must be disinfected prior to discharge at a production facility. Where feasible, this must include vehicle wheel dips at facility entry points and at locations where vehicles pass between identified disease risk zones within a hatchery. Vehicle and equipment disinfections at the hatcheries must be conducted at designated areas and must include external surfaces, empty internal tank surfaces, and equipment carried on the transportation units, such as nets, buckets, etc.

It is a common practice for employees from one facility to assist at another facility in order to complete certain tasks, such as cleaning a polishing lagoon or fin clipping. In these types of situations, special consideration must be given to disinfecting personal equipment and apparel, such as boots, outer weather gear, gloves, etc., that are exposed to fish or transport/receiving waters. This equipment must be disinfected before entering the water or handling fish. There must be several pairs of spare waders, aprons, and gloves on site for use by visiting workers. Occasionally, construction crews may need to have contact with water sources or production water in the performance of their duties. Their tools and personal equipment must be disinfected following the same protocols as hatchery staff. Felt-soled waders must not be used in hatchery waters.

Trucks or equipment that have been in contact with waters known to contain certain AIS such as zebra mussels and Didymo will undergo additional disinfection methods known to kill those species.

Care must be taken to avoid the discharge of potentially harmful, un-neutralized disinfectants.

D. Fish, Fish Gametes, and Fertilized Fish Egg Transfers

All transfers of fish gametes, fertilized eggs, and fish from within the PFBC fish production system and from other production sources must be approved in advance by the Fish Health Unit and the Fish Production Division Managers and Director.

6. Awareness

Training, oversight, and signage will be needed to maximize opportunities for success.

7. Summary

A biosecurity program can only be effective if it is a priority for administrators, hatchery managers and their staff, field biologists, regional outreach and education staff, water safety instructors, construction and maintenance operators, etc. Reduced flexibility will occur in moving fish from one facility to another to meet short-term production needs. Increased

awareness and vigilance will be needed to ensure that oversights or mishaps do not occur that could quickly undo years of biosecurity precautions. Staff from all PFBC divisions will be required to observe the biosecurity restrictions and measures defined in the individual hatchery management plans.

Appendix 1

A. Species-Specific Disinfectants and Procedures for Their Use

Note that many of these methods will be effective against multiple species – but when in doubt, always research which method is best for the particular species and equipment that is to be disinfected. Disinfection procedures for invertebrates are still being developed and evaluated. Thus, try to ensure successful disinfection – use the highest concentration disinfecting agent for the longest duration that won't adversely affect your gear. Always be aware of disposal procedures for disinfectant solutions in order to avoid accidentally polluting waterways!

Zebra/Quagga Mussel – *Dreissena spp.* (and most other invertebrates)

- Wash using a high temperature steam pressure washer at temperatures >200° F or 100°C for 3 – 10 minutes depending on organism lifestage, density, etc. (e.g., thick clusters of adults will take longer to kill than a few scattered larvae)
- Wash in water at a minimum temperature of 120° F (49° C) (e.g., undiluted hot tap water) for at least 20 minutes (note: water must be maintained at 120° F (49° C) or above throughout process)
- Use of chlorine-based disinfection procedures (see below) (precautions necessary)
- Equipment drying procedures (see below) – Note that it can take up to 21 days to kill adult zebra mussels by drying but most will die within one week (must be tested to confirm death)
- Phenol base cleaners (e.g., Lysol) – immersion in full strength for at least 2 minutes
- Ethanol (50%) – immerse for at least 2 minutes or use repeated flooding rinses of ethanol
- Salt solution (saturated salt solution diluted to 5%; e.g., 50 ml saturated salt solution in 950 ml water) – immersion for at least 30 minutes (exact exposure time depends on mussel life stage, density of mussels, etc.)
- Freezing solid for 1 – 24 hours depending on organism lifestage, density, etc.

Whirling Disease

- Wash using a high temperature steam pressure washer at temperatures >104° F or 40°C.

Didymo – *Didymosphenia geminata*

(** minimum of 1 minute exposure to any one (1) of the following):

- Hot water: 140°F
- Dishwashing detergent: 5% solution (~1 cup detergent to 1 gallon of water) ("environmentally friendly" detergents are not considered effective)
- Salt solution: 5% solution (saturated salt solution diluted to 5%; e.g., 50 ml saturated salt solution in 950 ml water)
- Air: *Didymosphenia geminata* can survive for months in moist conditions. If complete drying isn't possible, restrict use of gear to a single waterway.

Boats and Other Equipment – "Check, Clean, Dry"

- Check: Look for and remove visible algae and plant material from boots, gear, or anything that has made contact with the water or sediments.
- Clean: Soak, scrub, and/or expose all equipment in one of the solutions described above for a minimum of 1 minute. Absorbent items like felt-soled waders require 30-40 minutes of soaking.
- Air Dry: Items must be dried "to touch," and then allowed to dry for an additional 48 hours when possible.
    - *Didymosphenia geminate.* Dry: Items must be dried "to touch," and then allowed to dry for an additional 48 hours when possible. Can survive for months in moist conditions. If complete drying isn't possible, restrict use of gear to a single waterway.
    - Check trailers, trailer "bunks" with absorbent carpet, engines, paddles/oars, bilge areas, ropes, anchors, etc.

B. Disinfecting Solutions and Agents

Virkon: 0.5% (1:200) solution of Virkon Aquatic® sprayed on at an application rate of 300 ml per square meter. Virkon is available from Western Chemical. Contact number is 1-800-283-5292. PLEASE NOTE: Virkon Aquatic® is labeled for use only as a bactericide and viricide! Do not depend on its use against other AIS such as invertebrates (e.g. zebra mussel), plants, vertebrate species, etc. See above in Appendix 1 for other disinfection methods!

Chlorine: (NOTE: Chlorine, especially at high concentrations, is highly corrosive and causes degradation of plastics and rubber. Chlorine solutions must be neutralized with sodium thiosulfate prior to flushing.)

- 50% (1:1) household bleach (5.25% liquid sodium hypochlorite) dip, wipe, or spray; or
- 10% (1:9) household bleach (5.25% liquid sodium hypochlorite) immersion for 10 minutes; or
- 200 ppm [150 ml of household bleach (5.25% liquid sodium hypochlorite)/10 gal water or 35 g of granular 70% HTH® (pool chlorine)/26 gal water dip or spray (not for use on nets); or
- 20 ppm [15 ml of household bleach (5.25% liquid sodium hypochlorite)/10 gal water or 3.5 g of granular 70% HTH®/26 gal water complete immersion for 30 minutes.
- Household bleach (5.25% chlorine) can be purchased with VISA through the PFBC's cleaning supply contract (Grainger).
- HTH is granular chlorine (70% calcium hypochlorite) and can be purchased from a pool supply company.
- Sodium thiosulfate should be available at a pool supply company or from a chemical supply company.

Quaternary Ammonium Compounds (follow manufacturer instructions)

- Roccal-D™; or
- BrightWater™; or
- Parvosol™; or
- Formula 409®, 1:2 dilution for soaking or full strength as a spray for 10 minutes.

Heated Water

- 200°F (93°C) poured on gear
- 140°F (60°C) complete immersion for 15 minutes (requires a consistent heat source)
- 113°F (45°C) complete immersion for 60 minutes (requires a consistent heat source)

Salt Solution

- Always start with a saturated salt solution and dilute with water to the desired concentration (e.g., 5% salt solution; saturated salt solution diluted to 5%; 50 ml saturated salt solution in 950 ml water)

Sunlight

- Complete drying in direct sunlight for a minimum of 4-6 hours. Because of the necessarily limited times involved, this method is only recommended for non-absorbent materials.

Freezing

- Items must be frozen for a sufficient duration to kill all AIS life stages – preferably 24 hours or longer.

Air Drying

- Items must be dried long enough to completely dehydrate the organism of concern (many AIS can survive for months in barely damp conditions!). When in doubt, always dry to touch and then continue drying for at least an additional 48 hours. More absorbent materials will take more time to dry thoroughly.

Rubbing Alcohol (Ethanol)

- For wiping down small equipment.

Appendix 2

General Safety Precautions for Disinfectant Use

- When handling or spraying chlorine bleach solution, wear protective equipment (mask, gloves, goggles, rain gear, etc.) and use in a well-ventilated area (follow precautions on MSDS). Stay upwind when spraying. Chlorine will break down in sunlight and when in contact with organic material.

- Chlorine is corrosive to metal and rubber and is toxic to fish at the recommended concentrations. So, rinse well after disinfection or neutralize with sodium thiosulfate. For neutralizing chlorine, spray sodium thiosulfate at 800 ppm solution (3 grams per gallon of water) on all chlorine treated surfaces after the disinfection period is over. Rinse with water from the next waterbody to remove any remaining sodium spray.

- Virkon Aquatic – This is a disinfectant in the peroxygen (hydrogen peroxide) family. It is a powder. It is 99.9% biodegradable and breaks down to water and oxygen and is not corrosive at the working dilution. Wear a dust mask and eye protection if mixing powder. Wear rain gear and gloves if spraying. Stay upwind from the spray.

AIS Biosecurity Protocols Check List (08/08/08)

Equipment	Activity	Checked
AT WORK SITE		
INSPECT	Inspect and remove all visible aquatic plants, animals, mud, and other organic material from the boat, trailer, and equipment.	
DRAIN	Drain bilges or water holding containers.	
DISINFECT	Disinfect equipment.	
AT STORAGE FACILITY		
INSPECT	Inspect and remove all visible aquatic plants, animals, mud, and other organic material from the boat, trailer, or other equipment.	
DRAIN	Drain the bilges or other water holding equipment.	
DISPOSE	Collect disinfected and disposed of bilge water.	
DISINFECT	Operate pumps to take in the disinfectant and make sure that the solution comes in contact with all parts of the pump and hose.	
	Wash water holding compartments with a high temperature pressure washer or with an approved disinfectant.	
	Clean equipment used in field activities using a high temperature pressure washer or through application of disinfectant solution.	
Boat Motors		
INSPECT	Immerse the lower unit in a bucket of disinfectant and run the motor.	
DISINFECT	Allow the disinfectant to remain in the motor for the appropriate contact time.	
Large Equipment		
INSPECT	Inspect and remove all vegetation and other organic debris prior to disinfection.	
DISINFECT	Power washing is not required, but large equipment could be sprayed with a garden hose to remove debris.	
RINSE	After application of disinfectant solution, the equipment must be rinsed with clean water.	
Small Equipment & Personal Equipment		
INSPECT	Inspect and remove all organic material from gear.	
DISINFECT	Spray with disinfectant; maintain a wet surface for the appropriate contact time described in Appendix 1.	
RINSE	After application of disinfectant solution, the equipment must be rinsed with clean water.	

Appendix 3

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